REMARKS

Claim 36 is currently pending in this application and is in independent form. Claim 36 is believed to be in condition for allowance. Removal of the rejection and allowance of claim 36 is respectfully requested.

Claim 36 is directed to a method of preparing an osteogenic protein fraction, extracting demineralised bone matrix with a solution of at least one chaotropic agent; removing high molecular weight proteins which exceed 300 kDA from the extract by ultrafiltration with a 300 kDA membrane to produce a lower molecular weight fraction; subjecting the lower molecular weight fraction to heparin affinity chromatography under conditions which first favor the binding and then the elution of a purified heparin affinity fraction containing the osteogenic protein fraction; subjecting the heparin affinity fraction to hydroxyapatite chromatography under conditions which first favor the binding and then the elution of a purified osteogenic protein fraction; and exchanging the purified osteogenic protein fraction into a solvent suitable for human medical use. The chaotropic agent is selected from the group consisting of urea and guanidinium salts to produce an extract.

Claim 36 is rejected under 35 U.S.C. §103(a) as being obvious over either Scott et al. (1994, The Anatomical Record 238:23-30) (hereinafter "the Scott reference") or Yoshimura et al. (1993, Biol. Pharm. Bull. 16(5):444-447) (hereinafter "the Yoshimura reference") in view of United States Patent No. 4,968,590 to Kuberasampath et al. (hereinafter "the Kuberasampath patent").

The Scott reference is directed toward a method of isolated osteoinductive proteins from intramembraneous (IM) proteins from bones. [November 25, 2009 Dunea Declaration, paragraph 3]. The method includes extracting demineralised bone matrix with a solution of at least one chaotropic agent selected from the group consisting of urea and gaunidine salts (see left column of second page). [November 25, 2009 Dunea Declaration, paragraph 3]. The bone matrix proteins which are isolated have molecular weights of between 10 and 100 kDa (see left column of second page). [November 25, 2009 Dunea Declaration, paragraph 3]. A 100 kDa nominal molecular weight ultrafiltration membrane is used by the invention of Scott, in contrast to the claimed invention, which teaches the use of a 300 kDa nominal molecular weight

ultrafiltration membrane. [November 25, 2009 Dunea Declaration, paragraph 3]. The protein fraction is subjected to heparin affinity chromatography (see Abstract and left column of second page). [November 25, 2009 Dunea Declaration, paragraph 3]. The purified osteogenic protein fraction is then exchanged into a solvent suitable for human medical use. [November 25, 2009 Dunca Declaration, paragraph 3].

The Yoshimura reference is directed toward the purification of water-soluble bone-inductive protein from bovine demineralised bone matrix. [November 25, 2009 Dunea Declaration, paragraph 4]. The purification steps include ultrafiltration, dialysis, affinity chromatography on heparin-Sepharose and gel chromatography on Sephacryl S-200. [November 25, 2009 Dunea Declaration, paragraph 4]. The method also includes extracting demineralised bone matrix with a solution of at least one chaotropic agent selected from the group consisting of urea and gaunidine salts (see Introduction, second paragraph). [November 25, 2009 Dunea Declaration, paragraph 4]. The bone matrix proteins which are isolated have molecular weights of between 10 and 100 kDa (see Introduction, second last paragraph). [November 25, 2009 Dunea Declaration, paragraph 4]. A 100 kDa nominal molecular weight ultrafiltration membrane is used by Yoshimura, in contrast to the claimed invention, which teaches the use of a 300 kDa nominal molecular weight ultrafiltration membrane. [November 25, 2009 Dunea Declaration, paragraph 4]. The protein fraction is also subjected to heparin affinity chromatography (see Purification, step 3). The purified osteogenic protein fraction is then exchanged into a solvent suitable for human medical use. [November 25, 2009 Dunea Declaration, paragraph 4].

The Kuberasampath patent teaches a method of preparing an osteogenic protein fraction (see Abstract and column 1, lines 35-37). [November 25, 2009 Dunea Declaration, paragraph 5]. The method also includes extracting demineralised bone matrix with a solution of at least one chaotropic agent selected from the group consisting of urea and gaunidine salts (see column 6, lines 32- 52). [November 25, 2009 Dunea Declaration, paragraph 5]. The bone matrix proteins which are isolated have molecular weights greater than 10 kDa (see bottom of column 2 and top of column 3). [November 25, 2009 Dunea Declaration, paragraph 5]. A higher nominal molecular weight ultrafiltration membrane is not used by the invention of

Kubersampath, in contrast to the claimed invention, which teaches the use of a 300 kDa nominal molecular weight ultrafiltration membrane. [November 25, 2009 Dunea Declaration, paragraph 5]. The protein fraction, which is therefore not depleted of collagen, is also subjected to heparin affinity chromatography (see column 6, lines 53-68 and column 7, lines 1-9). [November 25, 2009 Dunea Declaration, paragraph 5]. In addition, Kuberasampath subjects the heparin affinity chromatography fraction to hydroxyapatite chromatography (see column 7, lines 10-32). [November 25, 2009 Dunea Declaration, paragraph 5].

Applicant asserts that the claimed invention is not obvious over the cited prior art since the claimed invention shows new and unexpected results relative to the prior art. An Applicant can rebut a presumption of obviousness "based on a claimed invention that falls within a prior art range by showing that there are new and unexpected results relative to the prior art." *Iron Grip Barbell Co., Inc. v. USA Sports, Inc.*, 392 F.3d 1317, 1322, 73 USPQ2d 1225, 1228 (Fed. Cir. 2004); MPEP 2144.05(III). Because the claimed method of preparing an osteogenic protein fraction using a 300 kDA membrane provides new and unexpected results relative to the Scott reference or the Yoshimura reference in view of the Kuberasampath patent; the claimed invention is not obvious over the cited prior art.

The comparative study clearly shows that the method of the claimed invention shows unexpected results over the prior art [November 25, 2009 Dunea Declaration, paragraph 9]. The Applicant accordingly submits that the use of a 300 kDa membrane to isolate a protein with a mass of 30-40 instead of a 100 kDa membrane produces a surprising increase in the yield of the pure protein. This choice was based on the Applicant's careful study of the processes which take place during the separation steps.

Particularly, to show that the method of the present invention produces an unexpectedly higher yield of pure BMP-2 than the methods of Scott, Yoshimura and Kuberasampath, the Applicant conducted side-by-side laboratory trials using a 100 kDa membrane and a 300 kDa membrane. [November 25, 2009 Dunea Declaration, paragraph 8, page 7]. The methods of the experiments are set forth in paragraph 8 of the November 25, 2009 Dunea Declaration.

The results of the comparative experiments show that almost twice the amount of BMP-2 was isolated in the 300 kDa fraction when compared to the crude affinity fraction and the 100 kDa fraction. [November 25, 2009 Dunea Declaration, paragraph 8 page 7]. The use of the 100 kDa membrane clearly shows that total protein recovery in the affinity fraction is poor, only 22% in comparison to the 300 kDa membrane. [November 25, 2009 Dunea Declaration, paragraph 8 pages 10-12]. The 100 kDa process produced only 55% of the amount of BMP-2 produced by the 300 kDa process. [November 25, 2009 Dunea Declaration, paragraph 8, pages 10-12]. The BMP-2 abundance (concentration) was highest in the 100 kDa method (5.1 ng/mAu versus 2.04 ng/mAu for 300 kDa method), but in commercial terms, the 300 kDa method will produce double the amount of implantable devices, as each device is dosed as a function of an amount of BMP-2 per implantable device. [November 25, 2009 Dunea Declaration, paragraph 8 pages 10-12]. Therefore, the claimed method of preparing an osteogenic protein fraction using a 300 kDA membrane provides new and unexpected results relative to the Scott reference or the Yoshimura reference in view of the Kuberasampath patent; and the claimed invention is not obvious over the cited prior art.

Additionally, the method of Kubersampath (patent 4, 968,590, page 6 lines 35-65) relies on ethanol precipitation of total protein, at the step prior to heparin Sepharose chromatography.). [November 25, 2009 Dunea Declaration, paragraph 8, page 13]. Ethanol precipitation with seven volumes of absolute ethanol will precipitate collagens and these will then be present during the next step of the Kubersampath process, which is heparin-Sepharose chromatography. [November 25, 2009 Dunea Declaration, paragraph 8, page 13].

The Applicant has found that contaminating collagens, and collagen aggregates in the initial extract from the demineralised bone matrix (DBM) interfere with the efficiency of the isolation of the bone morphogenetic proteins when using affinity chromatography. [November 25, 2009 Dunea Declaration, paragraph 8 pages 4-5]. The Applicant has found that the presence of high amounts of collagens and collagen aggregates typically co-extracted from DBM have a number of negative effects. [November 25, 2009 Dunea Declaration, paragraph 8 pages 4-5].

First, high amounts of collagens and collagen aggregates in the initial extract cause fouling of chromatographic columns. [November 25, 2009 Dunea Declaration, paragraph 8, pages 4-5]. This causes channeling phenomena, whereby proteins follow a path of least resistance through the affinity matrix, thereby bypassing binding sites on the chromatography media. [November 25, 2009 Dunea Declaration, paragraph 8, pages 4-5]. Additionally, the Applicant has found that the protein BMP-2 binds demineralised bone matrix collagen at low ionic strength conditions. [November 25, 2009 Dunea Declaration, paragraph 8, pages 4-5, Figures 2 and 3.]

In the method of the present invention, collagens and collagen aggregates are separated from BMP-2 at the beginning of the process at conditions of high ionic strength, using either I M NaCI solutions or 4 M guanidinium chloride solutions, prior to exchange in low ionic strength media (6M urea, 150 mM NaCI, 50 mM Tris-HCI, pH 7.4) and then applied onto heparin affinity chromatography media. [November 25, 2009 Dunea Declaration, paragraph 8, pages 4-5]. The Applicant has found that the competitive binding which takes place between BMP-2 and collagen, as demonstrated in Figure 2, is at least partly if not entirely responsible for BMP-2 losses during affinity chromatography on heparin chromatography media. [November 25, 2009 Dunea Declaration, paragraph 8 pages 4-5].

For at least the aforementioned reasons, the Applicant further submits that it would not have been obvious to a person skilled in the art to use a 300 kDa membrane if the derived protein BMP-2 has a mass of 30-40 kDa. The Applicant accordingly submits that the claimed invention is both new and non-obvious.

Additionally, the Examiner's view that the larger pore size would be expected to produce a higher yield of the BMP-2 protein, but that this would be at the expense of a reduction in purity is accordingly not supported by the commercial benefits of a higher total yield of BMP-2, within a milieu of a synergistic cocktail of multiple growth factors as demonstrated by Figure 6. [November 25, 2009 Dunea Declaration, paragraph 8 page 14].

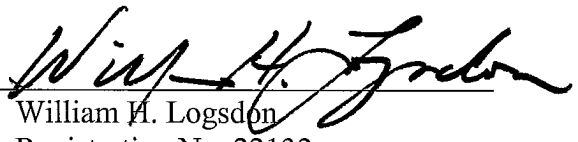
For at least the aforementioned reasons, the claimed method of preparing an osteogenic protein fraction using a 300 kDA membrane provides new and unexpected results relative to the Scott reference or the Yoshimura reference in view of the Kuberasampath patent.

CONCLUSION

For the foregoing reasons, Applicant submits that the Scott and Yoshimura references neither teach nor suggest the use of a 300 kDA filter in order to isolate the osteogenic protein fraction of the claimed invention and that the method of the present invention is accordingly both novel and nonobvious in view of the disclosures of Kuberasampath, Scott and Yoshimura. Reconsideration of the rejections and allowance of claim 36 is respectfully requested.

Respectfully submitted,

THE WEBB LAW FIRM

By 

William H. Logsdon
Registration No. 22132
Attorney for Applicant
436 Seventh Avenue
700 Koppers Building
Pittsburgh, PA 15219
Telephone: (412) 471-8815
Facsimile: (412) 471-4094
E-mail: webblaw@webblaw.com